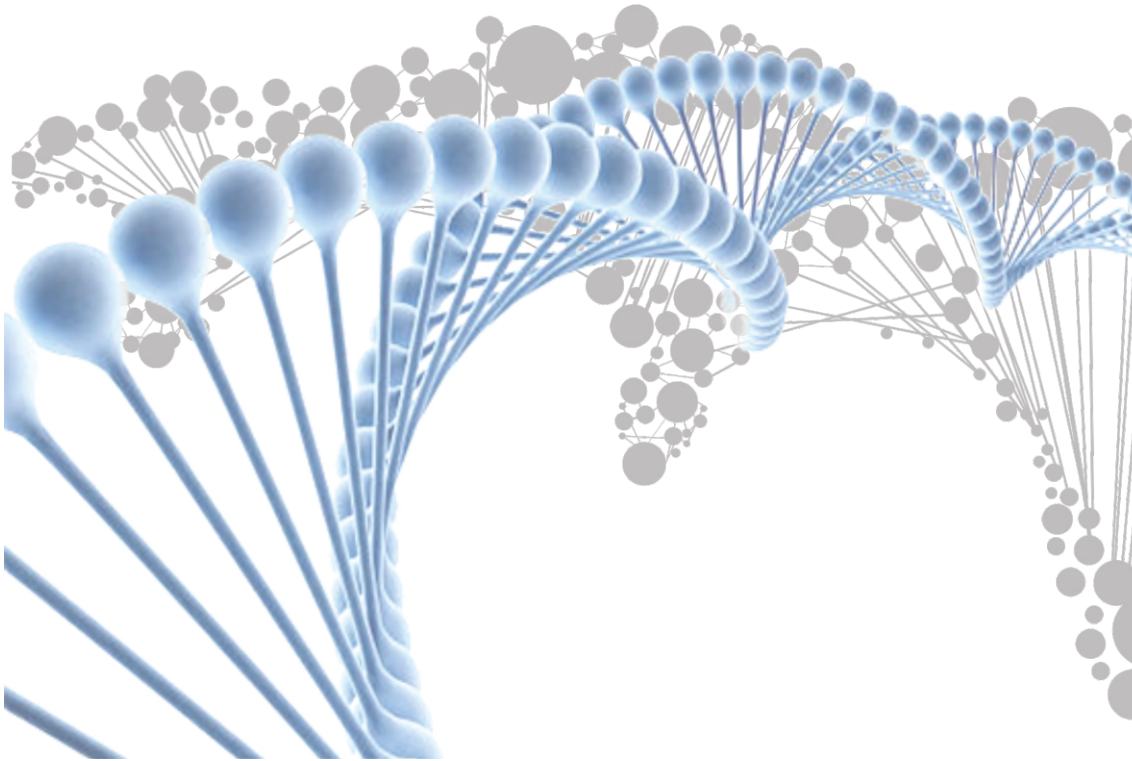
MPG-01100 & MPG-01300

Gel Extraction/ PCR Clean up Kit



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Gel Extraction & PCR Clean up Kit

Storage: Stored at room temperature (15-25°C).
Format: spin column **For research use only**
Sample: up to 300 mg of Agarose gel or 100 µl of PCR product.

Introduction:

The EBL Gel Extraction/ PCR Clean Up Kit provides a cost-effective system for fast and easy isolation of DNA fragments from PCR reactions, agarose gels, or enzymatic reactions. DNA fragments (100bp-10Kb) in specialized buffers are bound by the glass fiber matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and pure DNA is eluted with Tris buffer or water without phenol extraction or alcohol precipitation. DNA purified with the kits is suitable for any subsequent application, such as ligation and transformation, sequencing, restriction enzyme digestion, labeling, PCR, in vitro transcription, or microinjection. The entire procedure can be completed within 15-20 minutes.

About the kits:

Cat. No.	MPG-01100	MPG-01300
SP Columns	100 pcs	300 pcs
Collection Tubes	100 pcs	300 pcs
PG Buffer	60 ml	80 ml x2
W1 Buffer	45 ml	125 ml
W2 Buffer	15 ml	25 ml x2
Elution Buffer	10 ml	30 ml

Additional requirements:

* Ethanol (96~100%)

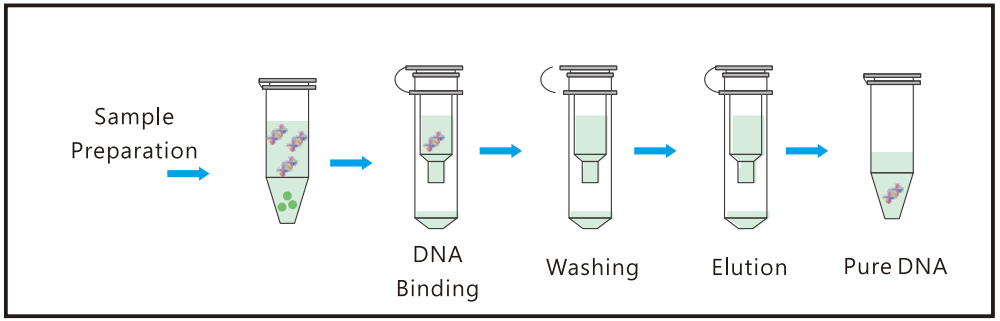
NOTE:

1. Add ethanol (96–100%) to Buffer W2 before use (see bottle label for volume).
2. If necessary, re-dissolve any precipitate by warming to 37°C.
3. Buffers PG and W1 contain irritants. Wear gloves when handling these buffers.

Related product and Ordering information:

CAT NO:	Product	Quantity
MGK-T0100	EBL Genomic DNA Isolation Kit (Tissue)	100 rxn.
MGK-T0300	EBL Genomic DNA Isolation Kit (Tissue)	300 rxn.
MGK-C0100	EBL Genomic DNA Isolation Kit (Cell/ Blood)	100 rxn.
MGK-C0300	EBL Genomic DNA Isolation Kit (Cell/ Blood)	300 rxn.
MGK-P0100	EBL Genomic DNA Isolation Kit (Plant)	100 rxn.
MGK-P0300	EBL Genomic DNA Isolation Kit (Plant)	300 rxn.
MPD-01300	EBL Plasmid Miniprep DNA Kit	300 rxn.
MPD-02025	EBL 200PLUS Plasmid Midiprep DNA Kit	25 rxn
MPD-03020	EBL 800PLUS Plasmid Maxiprep DNA Kit	20 rxn
FBRE100	Blood/ cell Total RNA Isolation kit	100 rxn.
FBRT100	Tissue Total RNA Isolation kit	100 rxn.
FBRP	Plant Total RNA Isolation kit	100 rxn.

Procedure:



Protocol:

Step 1. Sample Preparation

Gel Extraction

> Excise the DNA fragment from the agarose gel. Transfer up to 300 mg of the gel to a 1.5 ml microcentrifuge tube.
> Add 500 µl PG Buffer to the sample and mix by vortex.
> Incubate at 60℃ for 10 minutes (or until the gel slice has completely dissolved). During the incubation, mix by vortexing the tube every 2–3 minutes. Cool the dissolved sample mixture to room temperature.

PCR Clean up/ Enzymatic reactions Clean up

> Add 500 µl PG Buffer to 100 µl of the PCR reaction or enzymatic reactions and mix by vortex.

Step 2. DNA Binding

> Place a SP Column in a 2 ml Collection Tube.
> Apply the supernatant (from step 1) to the SP column by decanting or pipetting.
> Centrifuge at 14,000 x g for 30 seconds. Discard the flow-through and place the SP column back into the same Collection tube. (The maximum volume of the SP column reservoir is 700 µl. If the sample mixture is more than 700 µl, repeat the DNA Binding Step).

Step 3. Wash

> Add 400 µl of W1 Buffer into the SP Column. Centrifuge at 14,000 x g for 30 seconds.
> Discard the flow-through and place the SP Column back into the same Collection tube.
> Add 600 µl of W2 Buffer (Ethanol added) into the SP Column. Centrifuge at 14,000 x g for 30 seconds.
> Discard the flow-through and place the SP Column back into the same Collection tube.
> Centrifuge at 14,000 x g again for 2 minutes to remove residual W2 Buffer.

Step 4. DNA Elution

> Transfer the dried SP Column to a new 1.5 ml microcentrifuge tube.
> Add 50-200 µl of Pre-Heated Elution Buffer or TE into the center of the column matrix and let stand for 3 minutes.
> Centrifuge for 2 minutes at 14,000 x g to elute the purified DNA.

Troubleshooting

Problem	Comments and suggestions
Low yield of DNA	
>PG Buffer with the incorrect ratio added to the DNA product.	Verify that an correct volume of the PG Buffer was added to the reaction mixture.
>Ethanol not added	Add absolute ethanol (see the bottle label for volume) to the W2 Buffer prior to use.
>Nuclease contamination	Decrease the loading volume. If overloaded , separate the reaction mixture into 2 columns. If the DNA fragments are more than 300mg, separate the gel slice into two microcentrifuge tubes.
>Dissolved incompletely	Increase time for the Gel Extraction Step until the agarose gel has completely dissolved. Use an equal volume of the PG Buffer and/ or use lowmelting-point agarose gels.
>Incorrect elution conditions	Ensure that the Elution Buffer or ddH2Ois added into the center of the SP Column.
>Recovery buffer volume too small	Increase the amount of the Elution Buffer to at least 50 µl for use.

Troubleshooting

Problem	Comments and suggestions
Inhibition of downstream enzymatic reactions >TE buffer used for DNA elution	Use ethanol to precipitate the DNA, or re-purify the DNA fragments and elute with nuclease-free water.
>Presence of residual ethanol in DNA	Remove the EtOH in the hood briefly. Following the Wash step, dry the SP Column with additional centrifugation at 14~16,000 x g for 2 minutes.
DNA passed through in the flow-through or wash fraction >Column overloaded	Check the loading volume. If overloaded , separate into two columns.
>Inappropriate salt or pH conditions in buffers	Ensure that any buffer prepared in the laboratory was prepared according to instructions.
Purified DNA floats out of wells while running in agarose gel >Traces of ethanol not completely removed from the column	Make sure that no residual ethanol remains in the membrane before elution DNA. Re-centrifuge if necessary.